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(54) Title: METHODS OF DIAGNOSING AND MODULATING AUTOIMMUNITY

(57) Abstract

The invention features methods of modulating, e.g., inhibiting or increasing, autoimmunity in a subject which includes administering to the subject an agent which modulates cathepsing S activity, e.g., proteolysis of the Ii chain. The invention also features methods of modulating isotype switching, methods of treating a subject having an autoimmune disorder (e.g., a cathepsin S deficiency), and methods of evaluating a treatment for its ability to modulate an immune response, e.g., an MHC class II–dependent response. In addition, the invention features methods of diagnosing an autoimmune disorder such as a cathepsin S deficiency.

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METHODS OF DIAGNOSING AND MODULATING AUTOIMMUNITY

Background of the Invention

The invention relates to methods for modulating autoimmunity, screening for compounds which modulate autoimmunity, and methods of diagnosing an autoimmune disorder.

Summary of the Invention

Major histocompatability complex (MHC) class II molecules acquire antigenic peptides after endosomal degradation of the invarient chain (Ii), which is an MHC class II-associated protein which blocks peptide binding. In normal individuals, the protease cathepsin S has been found to cleave the Ii protein. The Ii chain transiently occupies the groove of MHC class II molecules to form MHC II/Ii chain complexes. The Ii chain prevents premature binding of antigenic peptides to MHC class II.

For MHC class II to become available to antigenic peptides, the Ii chain is first digested by cathepsin S into smaller fragments known as CLIP peptides. The CLIP peptides are removed by another MHC class II-like molecule and allow antigenic peptides to associate with the MHC class II groove. After this event, the antigenic peptide/MHC class II complex moves to the cell surface, where it is presented to $CD4^+$ T-cells. The T-cells release cytokines that stimulate B-cells to produce IgM antibodies which mature into IgG antibodies. The cells then undergo isotype switching to produce highly antigen-specific antibodies, e.g., IgG_2 and IgG_3.

The present invention is based, in part, on the discovery that cells in which the activity of protease cathepsin S has been inhibited fail to completely process Ii. This results in a marked delay of MHC class II peptide loading. In addition, it has been discovered that although the numbers of B cells and T cells and the initiation of antibody response to an antigen appear normal, inhibition of cathepsin S activity can further lead to defective germinal center formation and defective isotype switching. These discoveries indicate that cathepsin activity is essential for the full range of normal immune response.

Accordingly, the invention features, a method of modulating autoimmunity in a subject comprising administering to the subject an agent which modulates cathepsin S activity. A cathepsin activity includes proteolysis of the Ii chain. In a preferred embodiment, autoimmunity is inhibited, e.g., reduced or prevented, by inhibiting cathepsin S activity. The activity of cathepsin S can be inhibited by administering to the subject an agent which inhibits cathepsin S so as to inhibit Ii chain proteolysis. Agents which can be used to inhibit cathepsin S activity include antisense cathepsin nucleic acid molecules, cathepsin-specific antibodies and cathepsin inhibitory molecules, e.g., peptides based on vinylsulfone. The agent is administered to the subject in a therapeutically effective amount such that autoimmunity is inhibited

In another preferred embodiment, autoimmunity is increased, e.g., in an experimental animal, by increasing cathepsin activity. The activity of cathepsin can be increased by administering to the subject an agent which increases cathepsin activity so as to increase Ii chain proteolysis. Agents which can be used to increase cathepsin activity include cathepsin S, nucleic acid molecules which encode cathepsin S or biologically active portions thereof. The agent is administered to the subject in a therapeutically effective amount such that autoimmunity is increased or enhanced.

Preferably, the method of modulating autoimmunity by modulating cathepsin S activity specifically modulates MHC class II-dependent immunity.

Another aspect of the invention features a method of modulating isotype switching in a subject. Isotype switching includes lymphocyte activation and proliferation in the context of antigen stimulation. Isotype switching, e.g., the production of specific antibodies, (e.g., IgG_{2a}, IgG_3 and IgE,) can be modulated by inhibiting or increasing cathepsin activity such that Ii chain proteolysis is inhibited or increased. By inhibiting Ii chain proteolysis, defective antigen presentation and sensitization can occur which can thereby lead to defective isotype switching. Thus, in a preferred embodiment, isotype switching is inhibited by administering to a subject a therapeutically effective amount of an agent which inhibits cathepsin S activity. Such agents include antisense cathepsin nucleic acid molecules, cathepsin-specific antibodies and cathepsin inhibitory molecules, e.g., peptides based on vinylsulfone.

In another preferred embodiment, isotype switching can be increased by increasing cathepsin activity such that Ii chain proteolysis is increased. By increasing Ii chain proteolysis, increased isotype switching, e.g., high affinity specific Ig production, can be achieved. In one embodiment, isotype switching is increased by administering to a subject a therapeutically effective amount of an agent which increases cathepsin activity. Agents which can be used to increase cathepsin activity include cathepsin S, nucleic acid molecules which encode cathepsin S, or biologically active portions thereof.

In another aspect, the invention features, a method of treating an autoimmune disorder, e.g., asthma, in a subject. The method includes administering to a subject having an autoimmune disorder (e.g., asthma) an agent which regulates cathepsin S activity. Disorders such as asthma are associated with high titers of harmful autoantibodies and IgE. By using an agent which regulates, e.g., inhibits cathepsin S activity, proteolysis of the Ii chain can be delayed. Although not wishing to be bound by theory, such a delay is believed to lead to defective antigen presentation and, thus, defective isotype switching. Therefore, by regulating cathepsin S activity, the persistence of harmful autoantibodies and IgE associated with autoimmune disorders such as asthma can be reduced or prevented. Agents which can be used to inhibit cathepsin S activity include antisense cathepsin nucleic acid molecules, cathepsin-specific antibodies and cathepsin inhibitory molecules, e.g., peptides based on vinylsulfone.

In another aspect, the invention features a method of evaluating a treatment, e.g., a compound, for its ability to modulate an immune response e.g., an MHC class II-dependent response. Compounds which can be evaluated using this method include, for example, peptides, peptidomimetics, small molecule and drugs. In a preferred embodiment, the efficacy of a compound is evaluated by ability of the compound to modulate cathepsin S activity. In one embodiment, the ability of a compound to modulate cathepsin S activity can be evaluated by detecting the presence or absence of the Ii chain on the surface of a cell. The presence or absence of Ii on the cell surface can be determined, for example, using labeled anti-Ii specific antibodies.

In a preferred embodiment, a treatment, e.g., a compound, can be evaluated for its ability to inhibit an immune response, e.g., an MHC class II-dependent response. By detecting the presence of Ii on a cell surface using, for example, a labeled Ii-specific

antibody, the ability of a compound to inhibit cathepsin S activity can be determined. The presence of the Ii chain on a cell surface serves as an indicator of the ability of a compound to inhibit cathepsin S activity, and thereby inhibit an immune response, e.g., an MHC class II-dependent response.

In another preferred embodiment, a treatment, e.g., a compound, can be evaluated for its ability to increase an immune response, e.g., an MHC class II-dependent response. Using, for example, a labeled Ii-specific antibody, the absence or presence of Ii on a cell surface can be determined. The absence of Ii on the surface of a cell which originally expresses Ii on its surface serves as an indicator of the ability of a compound to increase cathepsin S activity, and thereby increase an immune response, e.g., an MHC class II-dependent response.

Another aspect of the invention features a method of diagnosing an autoimmune disorder. In a preferred embodiment, the autoimmune disorder is an enzyme deficiency disorder, e.g., a cathepsin S deficiency. Using, for example, anti-Ii antibodies, preferably an anti-Ii antibody with a detectable label, the presence of the Ii chain on a cell surface can be used as an indicator of a cathepsin S deficiency in a subject.

The term "labeled" or "detectably labeled", with regard to an antibody, is intended to encompass direct labeling of the antibody by coupling (i.e., physically linking) a detectable substance to the antibody, as well as indirect labeling of the antibody by reactivity with another reagent that is directly labeled.

The terms "isotype switching" and "antibody class switching" are used interchangeably herein. These terms refer to the biological process in which activated B cells within the lymph node germinal centers undergo a process of somatic hypermutation of immunoglobulin (Ig) genes. This leads to antigen driven selection of high affinity antibodies at these sites. Cells which produce Ig that bind the antigen weakly or not at all become apoptotic.

"High affinity antibodies" as used herein refers to antibodies which include IgG_2, IgG_3, IgM, and IgE.

A "treatment", as used herein, includes any therapeutic treatment, e.g., the administration of a therapeutic agent or substance, e.g., a drug.

The terms "peptides" and "polypeptides" are used interchangeably herein.

Subject, as used herein, can refer to a mammal, e.g., a human, or to an experimental animal or disease model. The subject can also be a non-human animal, e.g., a horse, cow, goat, or other domestic animal.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claim.

Detailed Description

Antisense Cathepsin Nucleic Acid Molecules

In one embodiment, the activity of cathepsin S, e.g., Ii chain proteolysis, is inhibited by introducing into a cell a nucleic acid which is antisense to a cathepsin S gene, thereby repressing transcription of the gene or translation of the mRNA. An "antisense" nucleic acid molecule comprises a nucleotide sequence which is complementary to a coding strand (i.e., sense strand) of another nucleic acid, e.g. complementary to an mRNA sequence, constructed according to the rules of Watson and Crick base pairing, and thus can hydrogen bond to the sense strand of the other nucleic acid. An antisense nucleic acid can form a duplex with an mRNA strand and prevent its efficient translation. Additionally, antisense nucleic acids may increase RNase-mediated degradation of mRNA and/or inhibit splicing of pre-mRNA. An antisense sequence can be complementary to a sequence found in the coding region of an mRNA or can be complementary to a 5' or 3' untranslated region of the mRNA. To inhibit translation, the antisense nucleic acid is preferably complementary to a region preceding or spanning the translation initiation codon. Alternatively, an antisense nucleic acid can bind to DNA to form a triple helix and prevent gene transcription (see e.g., Stein, C.A. and Cheng Y-C. (1993) *Science* 261:1004-1012). Thus, an antisense nucleic acid can be complementary in sequence to a regulatory region of a gene encoding cathepsin S, for instance complementary to a transcription initiation sequence or regulatory element (e.g., promoter or enhancer sequence). For a discussion of the regulation of gene expression

using antisense genes see Weintraub, H. et al., Antisense RNA as a molecular tool for genetic analysis, *Reviews - Trends in Genetics*, Vol. 1(1) 1986.

In one embodiment, a nucleic acid which is antisense to a regulatory or coding region of a cathepsin S gene is an oligonucleotide. Typically, oligonucleotides between about 5 and 50 nucleotides in length are used. More preferably, oligonucleotides between about 5 and 35 nucleotides are used. Even more preferably, oligonucleotides about 20 nucleotides in length are used. An antisense oligonucleotide can be constructed using chemical synthesis procedures known in the art. An oligonucleotide can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the oligonucleotide or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids. For example, the use of phosphorothioate, methyl phosphonate and ethyl phosphotriester antisense oligonucleotides (reviewed in Stein , C.A. and Cheng Y-C. (1993) *Science* 261:1004-1012) is within the scope of the invention. Additionally, acridine substituted nucleotides can be incorporated into the antisense oligonucleotides used in the present invention. Antisense oligonucleotides can be used to inhibit the activity of cathepsin S in a cell by genetic therapy and/or exogenously administering them to a subject at an amount and for a time period sufficient to inhibit transcription of the cathepsin S gene or translation of the cathepsin S mRNA in the cell.

In one embodiment, an antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (i.e., nucleic acid transcribed from the inserted sequence will be in an antisense orientation relative to a target nucleic acid of interest). The antisense expression vector is introduced into cells, for example, in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region of the vector, the activity of which can be determined by the cell type into which the vector is introduced. Preferably, the recombinant expression vector is a recombinant viral vector, such as a retroviral, adenoviral or adeno-associated viral vector. Protocols for producing recombinant retroviruses and for infecting cells *in vitro* or *in vivo* with such viruses can be found in Current Protocols in Molecular Biology, Ausubel, F.M. et al. (eds.) Greene Publishing Associates, (1989), Sections 9.10-9.14 and other standard laboratory manuals. Examples of suitable retroviruses include pLJ, pZIP, pWE and pEM

which are well known to those skilled in the art. Examples of suitable packaging virus lines include ψCrip, ψCre, ψ2 and ψAm. Adenoviral vectors are described in Berkner et al. (1988) *BioTechniques* 6:616; Rosenfeld et al. (1991) *Science* 252:431-434; and Rosenfeld et al. (1992) *Cell* 68:143-155. Suitable adenoviral vectors derived from the adenovirus strain Ad type 5 dl324 or other strains of adenovirus (e.g., Ad2, Ad3, Ad7 etc.) are well known to those skilled in the art. Adeno-associated vectors (AAV) are reviewed in Muzyczka et al. *Curr. Topics in Micro. and Immunol.* (1992) 158:97-129). An example of a suitable AAV vector is described in Tratschin et al. (1985) *Mol. Cell. Biol.* 5:3251-3260.

A recombinant expression vector containing a nucleic acid in an antisense orientation is introduced into a cell to generate antisense nucleic acids in the cell to thereby inhibit the activity of cathepsin S in the cell. The vector can be introduced into a cell by a conventional method for introducing nucleic acid into a cell. When a viral vector is used, the cell can be infected with the vector by standard techniques. Cells can be infected *in vitro* or *in vivo*. When a non-viral vector, e.g., a plasmid, is used, the vector can be introduced into the cell by, for example, calcium phosphate precipitation, DEAE-dextran transfection, electroporation or other suitable method for transfection of the cell.

In yet another embodiment, an antisense nucleic acid used to inhibit cathepsin S activity in a cell is a ribozyme which is capable of cleaving a single-stranded nucleic acid encoding cathepsin S, such as an mRNA transcript. A catalytic RNA (ribozyme) having ribonuclease activity can be designed which has specificity for an mRNA encoding cathepsin S. For example, a derivative of a Tetrahymena L-19 IVS RNA can be constructed in which the base sequence of the active site is complementary to the base sequence to be cleaved in a cathepsin S mRNA. See for example Cech et al. U.S. Patent No. 4,987,071; Cech et al. U.S. Patent No. 5,116,742 for descriptions of designing ribozymes. Alternatively, a cathepsin S RNA can be used to select a catalytic RNA having specific ribonuclease activity against the cathepsin S RNA from a pool of RNA molecules. See for example, Bartel, D. and Szostak, J.W. *Science* 261:1411-1418 (1993) for a description of selecting ribozymes. A ribozyme can be introduced into a cell by constructing a recombinant expression vector (e.g., a viral vector as discussed above) containing nucleic acid which, when transcribed, produces the ribozyme (i.e., DNA encoding the ribozyme is cloned into a recombinant expression vector by conventional techniques).

A preferred antisense nucleic acid of the invention is antisense to a coding or regulatory region of a cathepsin S gene. Antisense oligonucleotides, or an antisense recombinant expression vector can be designed based upon nucleotide sequences of cathepsin S cDNAs known in the art. The nucleotide sequence of a human cathepsin S cDNA is disclosed in Wienderanders, B. et al. (1992) *J. Biol. Chem.* 267(19):13708-13713, and Shi, G.P. et al. (1994) *J. Biol. Chem.* 269(15):11530-11536. In addition, the nucleotide sequence of a bovine cathepsin S cDNA is disclosed in Wiederanders, B. et al. (1991) *FEBS Lett.* 286(1-2):189-192, and the nucleotide sequence of a murine cathepsin S cDNA is available from Genbank™ (Accession Number AF051726). To inhibit the activity of cathepsin S in a cell from other species, antisense oligonucleotides are designed which are complimentary to nucleotide sequences that are conserved among cathepsin S genes in different species (e.g., based upon comparison of the known cathepsin S sequences, including the human, bovine and murine sequences, to identify conserved regions). Additionally, the known cDNA sequences can be used to design hybridization probes or PCR primers which allow isolation of cDNA and/or genomic DNA clones of cathepsin S from other species (e.g., pig) by standard techniques. An antisense nucleic acid for use in the invention can then be designed based upon the nucleotide sequence of a cDNA or genomic DNA fragment so isolated.

The pharmaceutical preparation of the gene therapy construct can consist essentially of the gene delivery system in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery system can be produced in tact from recombinant cells, e.g. retroviral vectors, the pharmaceutical preparation can comprise one or more cells which produce the gene delivery system.

Antibodies

The invention also includes methods of inhibiting autoimmunity and/or isotype switching by administering antibodies specifically reactive with cathepsin S. Anti-protein/anti-peptide antisera or monoclonal antibodies directed to cathepsin S can be made by standard protocols (See, for example, *Antibodies: A Laboratory Manual* ed. by Harlow and Lane (Cold Spring Harbor Press: 1988)).

Moreover, anti-Ii chain antibodies can be used diagnostically in ELISA, FACS analysis, and standard imaging techniques to detect and evaluate Ii chain expression *in vitro* or *in vivo*.

Intact antibodies, or fragments thereof (e.g., Fab or $F(ab')_2$) can be used in the present invention. Antibody fragments can be made by standard protocols known in the art.

The antibodies can be used to monitor Ii protein levels as part of a clinical testing procedure, e.g., to, for example, determine the efficacy of a given treatment regimen or to diagnose autoimmune disorders. Detection can be facilitated by coupling (i.e., physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{35}S or ^{3}H.

Cathepsin S Inhibitors

Peptides Based on Vinylsulfone

This invention includes methods of inhibiting autoimmunity and/or isotype switching by administering a cathepsin S inhibitor, e.g., a peptide based on vinylsulfone. Peptide vinylsulfone compounds include asparaginyl- leucyl-vinylsulfone, arginyl-methionyl-vinylsulfone, leucyl-arginyl-methionyl-vinylsulone, glutamyl-asparaginyl-leucyl-vinylsulfone, and leucyl-leucyl-leucyl-vinylsulfone. Modifications of these peptide vinylsulfones are also included as cathepsin S inhibitors in the present invention. For example, carboxybenzyl can be present at the N-terminal end to give the following compounds:

N-(carboxybenzyl)-asparaginyl-leucyl-vinylsulfone, N-(carboxybenzyl)-arginyl-methionyl-vinylculfone, N-(carboxibenzyl)-leucyl-arginyl-methionyl-vinylsulfone, N-(carboxybenzyl)-glutamyl-asparaginyl-leucyl-vinylsulfone, and N-(carboxybenzyl)-leucyl-leucyl-leucyl-

vinylsulfone. In an alternative, nitrophenylacetyl is present at the N-terminal end to give the following compounds: N-(nitrophenylacetyl)-asparaginyl-leucyl-vinylsulfone, N-(nitrophenylacetyl)-arginyl-methionyl-vinylsulfone, N-(nitrophenylacetyl)-leucyl-arginyl-methionyl-vinylsulfone, N-(nitrophenylacetyl)-glutamyl-asparaginyl-leucyl-vinylsulfone and N-(nitrophenylacetyl) leucyl-leucyl-leucyl-vinylsulfone. A peptide based vinylsulfone is meant to include, e.g., a peptide vinylsulfone or a modified peptide vinylsulfone. Other modifications of the peptide based vinylsulfones, e.g., substitutions can be added at the amino terminus of the peptide-based vinylsulfones by, e.g., N-methyl substituents or any other alkyl or substituted alkyl chain, or by substitution with, e.g., phenyl, benzyl, aryl, or modified aryl substituents, as would be known to those skilled in the art and are encompassed by the present invention as cathepsin inhibitors.

Peptide based vinylsulfones used as cathepsin inhibitors in the present invention can also correspond to a preferred cleavage site of the protease cathepsin on the invariant chain (Ii) polypeptide. These major cleavages occur at the C-terminal of the arginine and lysine, respectfully, in the amino acid sequence described in PCT publication WO97US/6865, published on October 30, 1997.

Pharmaceutical Compositions and Dosages

Administration of an agent can be accomplished by any method which allows the agent to reach the target cells, e.g., MHC class II antigen presenting cells. These methods include, e.g., injection, infusion, deposition, implantation, suppositories, oral ingestion, inhalation, topical administration, or any other method of administration where access to the target cells by the agent is obtained. For example, agents used in the present invention can be administered by injection, e.g., intravenous, intradermal, subcutaneous, intramuscular or intraperitoneal. In certain embodiments, the injections can be given at multiple locations. Implantation includes inserting implantable drug delivery systems, e.g., microspheres, hydrogels, polymeric reservoirs, cholesterol matrices, polymeric systems, e.g., matrix erosion and/or diffusion systems and non-polymeric systems, e.g., compressed fused or partially fused pellets. Suppositories include glycerin suppositories. Oral ingestion doses can be enterically coated. Inhalation includes administering the agent with an aerosol in an inhalator, either alone or attached to a carrier which can be absorbed.

In certain embodiments or the invention, the administration can be designed so as to result in sequential exposures to the agent over some time period, e.g., hours, days, weeks, months or years. This can be accomplished by repeated administrations of the agent by one of the methods described above, or alternatively, by a controlled release delivery system in which the agent is delivered to the mammal over a prolonged period without repeated administrations. By a controlled release delivery system is meant that total release of the agent does not occur immediately upon administration, but rather is delayed for some time period. Release can occur in bursts or it can occur gradually and continuously. Administration of such a system can be, e.g., by long acting oral dosage forms, bolus injections, transdermal patches and subcutaneous implants.

Examples of systems in which release occurs in bursts include, e.g., systems in which the agent is entrapped in liposomes which are encapsulated in a polymer matrix, the liposomes being sensitive to a specific stimuli, e.g., temperature, pH, light or a degrading enzyme, and systems in which the agent is encapsulated by an ionically-coated microcapsule with a microcapsule core-degrading enzyme. Examples of systems in which release of the agent is gradual and continuous include, e.g., erosional systems in which the agent is contained in a form within a matrix, and diffusional systems in which the agent permeates at a controlled rate, e.g., through a polymer. Such sustained release systems can be, e.g., in the form of pellets or capsules.

The agent can be suspended in a liquid, e.g., in dissolved form or colloidal form. The liquid can be a solvent, partial solvent or non-solvent. In many cases water or an organic liquid can be used.

The agent can be administered prior to, or subsequent to, onset of the disorder or disease.

The agent is administered to the subject in a therapeutically effective amount. A "therapeutically effective amount" refers to the amount which is capable of, at least partially, preventing or reversing the particular automimmune disease. A therapeutically effective amount can be determined on an individual basis and will be based, at least in part, on consideration of the species of mammal, the mammal's size, the agent used, the type of delivery system used, the time of administration relative to the severity of the disease, and

whether a single, multiple, or a controlled release dose regimen is employed. A therapeutically effective amount can be determined by one of ordinary skill in the art employing such factors and using no more than routine experimentation.

Preferably, the concentration of the agent if administered systematically is at a dose of about 1.0 mg to about 2000 mg for an adult of 70 kg body weight, per day. More preferably, the dose is about 10 mg to about 1000 mg/70 kg/day. Most preferably, the dose is about 100 mg to about 500 mg/70 kg/day. A preferred dosage range for administration of an antibody to a human subject is about 0.1-0.3 mg/kg of body weight per day. The specific concentration partially depends upon the particular agent used, as some are more effective than others. The dosage concentration of the agent that is actually administered is dependent at least in part upon the particular automimmune disease being tested, the final concentration of agent that is desired at the site of action, the method of administration, the efficacy of the particular agent, the longevity of the particular agent, and the timing of administration relative to the severity of the disease. Preferably, the dosage form is such that it does not substantially deleteriously affect the subject. The dosage can be determined by one of ordinary skill in the art employing such factors and using no more than routine experimentation.

Screening Assays for Evaluating a Treatment

The invention also provides a method (also referred to herein as a "screening assay") for evaluating a treatment, e.g., test compounds (e.g., peptides, peptidomimetics, small molecules or other drugs) which modulate cathepsin S activity and, thereby have a stimulatory or inhibitory effect on the expression of the Ii chain on a cell surface.

Test compounds which can be used in the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, K.S. (1997) *Anticancer Drug Des.* 12:145).

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt et al. (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:6909; Erb et al. (1994) *Proc. Natl. Acad. Sci. USA* 91:11422; Zuckermann et al. (1994). *J. Med. Chem.* 37:2678; Cho et al. (1993) *Science* 261:1303; Carrell et al. (1994) *Angew. Chem. Int. Ed. Engl.* 33:2059; Carell et al. (1994) *Angew. Chem. Int. Ed. Engl.* 33:2061; and in Gallop et al. (1994) *J. Med. Chem.* 37:1233.

Libraries of compounds may be presented in solution (e.g., Houghten (1992) *Biotechniques* 13:412-421), or on beads (Lam (1991) *Nature* 354:82-84), chips (Fodor (1993) *Nature* 364:555-556), bacteria (Ladner USP 5,223,409), spores (Ladner USP '409), plasmids (Cull et al. (1992) *Proc Natl Acad Sci USA* 89:1865-1869) or on phage (Scott and Smith (1990) *Science* 249:386-390); (Devlin (1990) *Science* 249:404-406); (Cwirla et al. (1990) *Proc. Natl. Acad. Sci.* 87:6378-6382); (Felici (1991) *J. Mol. Biol.* 222:301-310); (Ladner *supra.*).

In one embodiment, a screening assay comprises contacting a cell, i.e., an antigen presenting cell, which expresses cathepsin S, with a test compound and determining the ability of the test compound to modulate (e.g. stimulate or inhibit) cathepsin S activity. Determining the ability of the test compound to modulate the activity of cathepsin S can be accomplished, for example, by determining the absence or presence of the Ii chain on the surface of the cell. The absence or presence of the Ii chain on the surface of the cell can be determined, for example, using a labeled antibody which binds specifically to the Ii chain and detecting the labeled antibody.

Compounds identified by the above-described method can further be used in an appropriate animal model. For example, compounds identified and evaluated as described herein can be used in an animal model to determine the efficacy, toxicity, or side effects of treatment with such a compound.

Monitoring of Effects During Clinical Trials to Evaluate a Treatment

Monitoring the influence of a treatment (e.g., a compound) on cell surface expression of Ii can be applied not only in basic drug screening, but also in clinical trials. For example, the effectiveness of a compound determined by a screening assay as described herein to inhibit cathepsin S activity and thereby increase Ii cell surface expression, can be

monitored in clinical trials of subjects exhibiting an increased immune response, e.g., an MHC class II-dependent response. Alternatively, the effectiveness of an agent determined by a screening assay to enhance cathepsin S activity and thereby reduce or eliminate Ii cell surface expression, can be monitored in clinical trials of subjects exhibiting decreased immune response, e.g., an MHC class II-dependent response.

In a preferred embodiment, the present invention provides a method for evaluating, e.g., monitoring, the effectiveness of treatment of a subject with a compound (e.g., peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate identified by the screening assays described herein) comprising the steps of (i) evaluating pre-administration levels of cathepsin S activity in a subject prior to administration of a compound by detecting the level of Ii expression, e.g., the absence or presence of Ii expression, on cells of the subject; (ii) administering a compound to the subject; (iii) evaluating the level of cathepsin S activity post-administration of the compound by detecting the level of Ii expression on cells of the subject; and (iv) comparing the level of Ii expression prior to and subsequent to the administration of the compound. According to such an embodiment, the expression of the Ii chain on cells of the subject may be used as an indicator of the effectiveness of a compound.

Diagnostic Assays

The diagnostic method of the invention can be used to diagnose a cathepsin S deficiency by detecting Ii chain expression on the surface of cells *in vitro* as well as *in vivo*. A preferred indicator of Ii expression is an antibody capable of binding to the Ii chain, preferably an antibody with a detectable label. *In vitro* techniques for the detection of the Ii chain include, for example, enzyme linked immunosorbent assays (ELISAs), FACS analysis, and immunohistochemical analysis. In addition, *in vivo* techniques for detecting Ii include introducing into a subject a labeled anti-Ii antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

EXAMPLES

Example I: Development of "Knockout" Mice Deficient in Cathepsin S

To establish the role of cathepsin S in Ii processing and MHC class II antigen presentation *in vivo*, murine cathepsin S gene was targeted by homologous recombination and MHC class II function was characterized in the resulting mutant mice.

Exon 5, which contains the active site cysteine of murine cathepsin S, was deleted by recombination with a targeting vector in ES 129 cells and mice homozygous for mutant cathepsin S were generated in a MHC class II I-A^b background. Briefly, an Apa 1 fragment of a genomic P1 clone containing the murine cathepsin S gene (Genome Systems, St. Louis MO) was used to generate a 2.3 kilobase (kb) DNA fragment containing exon 4 and a 6 kb fragment containing exon 6. Both sequences were subcloned into *BamH I* sites before and after a neomycin resistant gene (NEO) of the pPNT targeting vector (Dranoff et. al. *Science* 264,173 [1994]). The NEO cassette replaced the active cysteine site within exon 5 (Shi et. al. (1994) *J. Biol. Chem.* 269:11530). The resulting targeting vector was linearized with *Not I* digestion and used to transfect D3 ES cells (129/SJ) followed by selections with both 300 μg/ml G418 and 2 μM gangcylovir. The positive ES cell clones, as confirmed by Southern blot analysis, were used to inject 3.5-day-old blastocysts. Germline transmission into C57bl/6 mice was assessed by Southern blot analysis of agouti offsprings. Both targeting vector transfected ES cell clones and the progeny mouse genotypes were determined by digesting genomic DNA with *NdeI* and *sa1I* followed by probing with an external genomic DNA fragment.

Example II: Evaluation of Cathepsin S Expression

Splenocytes from these mice were used to determine whether cathepsin S protein was present in these mice by immunoblotting or by active site labeling with ^{125}I- Z-tyr-ala-diazomethylketone, an irreversible inhibitor of cysteine protease.

Briefly, mouse splenocytes were isolated by mechanically teasing the spleen followed by red blood cell depletion. Five million cells were lysed into 150 μl of sample buffer, separated onto 12% SDS-PAGE, blotted onto nitrocellulose membrane, and probed with polyclonal antisera against recombinant mouse cathepsin S. The antiserum was raised by immunization of rabbits with a fusion protein comprised of maltose binding protein (pMAL-cRI, New England Biolabs, Beverly MA) and 150 residues of murine cathepsin S extending between the active site cysteine and asparagine residues. Resulting antisera were purified by elution from nitrocellulose-bound fusion protein and verified by western blotting

to react with murine cathepsin S but not other cysteine proteases as described in Reise, R.J. (1996) *Immunity* 4:357. The results demonstrate that spenocytes from these mice contained no detectable cathepsin S protein.

Sections of lymph node, spleen, and thymus failed to stain with anti-cathepsin S antibodies, whereas, staining of macrophage-like cells was observed in all tissues examined of wild type mice. Single cell suspensions were incubated at 4°C for 30 minutes with appropriate mAb coupled to fluorescent moieties in the presence of Fc block (Pharmingen, San Diego, CA), washed, and fixed in 1% paraformaldehyde. Samples were analyzed on a FACScan (Becton Dickinson, San Jose, CA). Cathepsin S -/- mice have normal fertility and up to 3 months in microisolators appear normal. FACS analyses of thymic, lymph node, and splenic lymphocytes from 1-2 month old animals revealed normal ratios of B220+ B cells and CD4+ and CD8+ T cells. T cells were purified from cathepsin S +/+, +/-, and -/- splenocytes using nylon columns according to the manufacturer (Polysciences, Inc) followed by repeated MHC class II antibody (Pharmingen) and complement (Accurate Chemical Co) depletions. Such T cells and unfractionated splenocytes from the same mice or from Balb/c mice (I-Ad) were used as responders at 2 x 10^5 cells per microtiter well. Single cell splenocyte suspensions were also prepared from the various mice, irradiated, and used as stimulators (0-8 x 10^5) in mixed lymphocyte reactions. Incubation in RPMI containing 10% fetal bovine serum, 1 pCi of [3H]-thymidine was added and 18 hrs later the cultures harvested (Tomtec) and incorporated thymidine assayed Betaplate reader (Wallac). At least five independent experiments in triplicate were performed for each allogeneic or syngeneic mixed lymphocyte reaction. Purified splenic T cells from -/- and +/+ mice proliferated equally in response to allogeneic stimulation. These results demonstrate that cathepsin S -/- mice do not show developmental abnormalities in their lymphocytes.

Example III: Analysis of Cathepsin S Activity on MHC class II Associated Ii

The effects of inhibiting cathepsin S activity on Ii degradation and MHC class II peptide loading were evaluated as follows:

Splenocytes were pulsed for 30 minutes with ^{35}S-methionine (New England Nuclear) followed by incubation in unlabeled medium for the indicated times as described in Villadangos, J.A. et al. (1997) *J. Exp. Med.* 186:549. Cells were then lysed into a buffer containing 50 mM Tris.HCl, pH 7.4, 0.5% NP-40, and mM $MgCl^2$. Protein concentration

was normalized to radioactivity of trichloroacetic acid precipitates. Equal radioactivity from each sample was precleared with normal rabbit serum and mouse serum and precipitated with MHC class II antibody. Both boiled (B) and nonboiled (NB) samples in sample buffer were separated by 12.5 % SDS-PAGE.

In splenocytes from normal mice, MHC class II-associated Ii was almost fully degraded and replaced with peptides within hours of synthesis. In contrast, MHC class II complexes in splenocytes from -/- mice retained an ~10 kDa fragment of Ii (Ii pl0), the identity of which was confirmed by immunoprecipitation with anti-Ii antibodies. Prior studies of Ii processing have shown that Ii pl0 is comprised of the region of Ii extending from its N-terminus through the C-terminus of CLIP (Villadangas, J.A. et al. (1997) *J. Exp. Med.* 186:549). Accumulation of MHC class II/Ii pl0 complexes coincided with a near total blockade of MHC class II peptide loading. Similar results were observed with unfractionated lymph node cells from cathepsin S-deficient mice and when the chase periods were extended to 24-48 hrs.

Example IV: Determination of MHC class II Cell Surface Expression

The effects of inhibited Ii degradation on MHC class II cell surface expression was determined as follows. Briefly, splenocytes were incubated for 20 minutes in ice-cold PBS containing 0.5 mg/ml NHS-Sulfo-Biotin (Pierce) at 4°C after cells were pulsed and chased as described in Example II. The biotinylated cells were lysed into NP-40 lysis buffer, precipitated with N22 for analysis of MHC class II processing, and precipitates transferred to nitrocellulose filters. Filters were developed with 1:1000 dilutions of peroxidase-conjugated avidin (Sigma) and subsequently exposed to Kodak XOMAT film to visualize metabolically labeled proteins.

Surprisingly, steady state surface expression of MHC class II molecules on splenocytes of -/- mice was not reduced significantly. Both FACS analysis and direct surface labeling of splenocytes followed by immunoprecipitation of MHC class II complexes showed surface expression of α/β heterodimers, approximately 75% (range 50-100% in five different experiments) that of normal littermates. Surface MHC class II complexes from splenocytes of -/- mice consisted of SDS-stable MHC class II/peptide complexes and complexes of MHC class II containing Ii pl0; the latter are absent from +/+ mice. In mixed lymphocyte reactions, proliferation of I-A^d splenocytes in response to

stimulation with splenocytes of cathepsin S -/-I-A^b mice was reduced ~50% compared to stimulation with cells from +/+ littermate controls, consistent with a significant fraction of surface I-A^b of -/- mice being occupied by Ii pl0 as demonstrated in Example II. Although peptide loading of newly synthesized MHC class II heterodimers is markedly delayed and Ii pl0 retained, many dimers ultimately acquire peptide, possibly in endocytic compartments other than MIIC/CIIV, and reach the cell surface.

Example V: Functional Analysis of the Effects of Reduced MHC class II Peptide Loading Rates

The effects of reduced MHC class II peptide loading rates were examined. Briefly, mice were immunized in footpads with a mixture of ovalbumin (100 μg) and HEL (100 μg) in complete Freund's adjuvant (CFA). Ipsilateral and contralateral draining lymph nodes were examined nine days later after fixing the nodes with 10% formalin and embedding in paraffin. Immunostaining was performed using avidin-biotin complexes (ABC) according to the manufacturer's instructions (Vector). Primary B cell follicles within the contralateral lymph nodes of -/- mice were consistently smaller than those of their +/+ littermates. Ipsilateral lymph nodes obtained from immunized mice of both +/+ and -/- mice were enlarged to a similar degree (3-5-fold) over the contralateral nodes. Microscopic examination revealed the expected numerous germinal centers in +/+ mice but the near complete absence of germinal centers in -/- mice, suggesting failure of local B cell activation and proliferation in these mice.

Tissue sections were dewaxed, dehydrated, blocked with non-specific serum and stained at 4°C overnight with biotinylated lectin peanut agglutinin (PNA) (Sigma) which binds to immature, proliferating B cells within germinal centers. Endogenous peroxidase activity was quenched using methanol containing 1% hydrogen peroxide. ABC were applied to the samples, and incubated at room temperature for 1 hour. Biotinylated tyramide was then added for 7 minutes followed by strepavidin-horseradish peroxidase (NEN). Immunopositivity was localized using chromagen diaminobenzidine (0.0251) in PBS and 0.1% hydrogen peroxide. PNA stained central follicular areas in +/+ mice but showed much weaker or no organized staining in lymph node follicles of -/- mice. Activated B cells within germinal centers undergo a process of somatic hypermutation of immunoglobulin (Ig) genes, leading to antigen-driven selection of high-affinity antibodies at

these sites. Cells which produce Ig that bind antigen weakly or not at all become apoptotic (Kelsoe (1997) *Semin. Immunol.* 8:179; and Pulendran et al. (1995) *Nature* 375:334).

Lymph nodes were assessed by assays for increased DNA strand breaks. Briefly, lymph node section apoptosis assays were performed using an Apop-Tag kit (Oncor) according to the manufacturer's instructions. The lymph node paraffin sections were digested with protease K, incubated with terminal deoxynucleotidyl transferase (TdT) enzyme, stained with anti-digoxigenin-fluorescein, and mounted with a propyiodine (2.5 μg/ml)/glycerol gel (Dako) mixture. The lymph nodes from the -/- mice were virtually devoid of organized apoptotic activity whereas clusters of apoptotic cells were widespread in the draining nodes of immunized +/+ mice. Thus, lymphocyte activation and proliferation in the context of antigenic stimulation *in vivo* is markedly depressed in the absence of cathepsin S. This interpretation is supported by results of functional assays of antigen presentation using unfractionated lymph node cells from the immunized mice. Lymph node cells were teased from the draining lymph nodes of mice foot pads immunized as described above. Cells ($8x10^5$ per microtiter well) were suspended in RPMI containing 10% FBS and incubated with various amounts of antigen (both OVA and HEL) for 48 hours followed by the addition of 1 μCi of [^{3}H]-thymidine. Thymidine incorporation was assessed after another 16 hours of incubation at 37°C. Proliferation of T lymphocytes obtained directly from immunized -/- mice was depressed following restimulation with antigens *in vitro* indicating defective *in vivo* presentation and sensitization.

Example VI: Assessment of Ig isotype Production

Activated B cells not only undergo somatic hypermutation and selection within germinal centers but also undergo class switching from IgM to IgG_1 as well as other isotypes, depending on the cytokine milieu in which activation occurs. Because germinal center development is defective in cathepsin S -/- mice, the profile of Ig isotypes produced by normal and mutant mice in response to immunization was also assessed. Wild type (+/+) and cathepsin S deficient (-/-) mice were immunized interpertoneally with 100 μg of both OVA and HEL in CFA and boosted with antigens in alum 7 days later. Mouse sera were collected before and 14 days after initial immunization and diluted into PBS containing 1% BSA and 0.05% Tween-20. The 96-well immuno plates (Nunc) were precoated overnight with 50 μl of 20 μg/ml either OVA or HEL. After one hour of blocking with 3% BSA, serial dilutions of sera were added onto the plates followed by overnight incubation at 4°C.

Plates were then washed and incubated with peroxidase conjugated secondary antibodies (Southern Biotech.) for another hour followed by washing and developing with OPD (Sigma). Whereas +/+ mice develop IgM and the expected range of IgG isotypes in response to either antigen, -/- mice show IgM responses comparable to +/+ mice but strongly diminished IgG responses. Markedly deficient (< 20% control) IgG_{2a} and IgG_3 responses to either antigen were observed in -/- mice.

Example VII: Analysis of Possible Factors involved with Defective Antigen Presentation

Defective antigen presentation in cathepsin S -/- mice could be due not only to impaired loading of MHC class II molecules with peptides but also to failure of APC to generate relevant peptides in the absence of cathepsin S. To examine the latter possibility, established T cell hybridomas derived from +/+ $I\text{-}A^b$ mice, and recognizing either an ovalbumin peptide (323-339) or a hen egg lysozyme (HEL) peptide (74-88), were exposed to antigen and APC from normal and mutant mice *in vitro*. Briefly, wild type (+/+) and cathepsin S deficient (-/-) mice were immunized intraperitoneally with 100 μg of both OVA and HEL in CFA and boosted with antigens in alum 7 days later. Mouse sera were collected before and 14 days after initial immunization and diluted into PBS containing 1% BSA and 0.05% Tween-20. The 96-well immuno plates (Nunc) were precoated overnight with 50 μl of 20 μg/ml either OVA or HEL. After one hour of blocking with 3% BSA, serial dilutions of sera were added onto the plates followed by overnight incubation at 4°C. Plates were then washed and incubated with peroxidase conjugated secondary antibodies (Southern Biotech) for another hour followed by washing and developing with OPD (Sigma).

Splenocytes from cathepsin S +/- mice performed similarly to those of normal mice and induced IL-2 secretion in response to either antigen in dose dependent manner. Splenocytes from homozygous mutants (-/-) failed to present effectively the ovalbumin peptide. In separate studies, it has been determined that inhibition of cathepsin S by a selective protease inhibitor does not block generation of peptide 323-339 in murine splenocytes but does block presentation to the same hybridoma. In contrast, APC from -/- mice presented the HEL epitope better than APC from +/+ mice. Thus, cathepsin S -/- APC have the intrinsic capacity to generate and even present certain antigenic epitopes despite retained Ii p10 in MHC class II dimers, an observation consistent with both enhancing and inhibiting effects of Ii on MHC class II peptide loading (Momburg, F. et al. (1993) *J. Exp. Med.* 178:1453; Peterson, M. et al. (1992) *Nature* 357:596). Although cathepsin S -/- APC

can present an HEL epitope to an established T cell hybridoma obtained from a +/+ animal, it is likely that their failure to present effectively *in vivo* reflects a more stringent requirement for quantity and diversity of peptides displayed by APC to initiate a normal immune response.

Results

Cathepsin S activity is essential for the full range of normal MHC class II-dependent immunity. The mechanism by which cathepsin S promotes immunity is potentially explained by its critical influence on the rate of peptide loading of MHC class II molecules. As antigenic peptides are continuously being generated and degraded within endosomal/lysosomal compartments, efficient capture and presentation of antigens requires MHC class II dimers competent for peptide loading in the right place at the right time. In the absence of cathepsin S, the timing of these events is altered. Peptide loading by MHC class II/Ii p10 is slow and likely limited to those peptide epitopes which are resistant to rapid degradation and capable of being loaded in the presence of retained Ii p10. Trafficking of MHC class II/Ii p10 within APC may also be altered (Brachet et al. (1997) *Cell Biol.* 137:51). That peptide loading nonetheless occurs in this setting is indicated both by the ability of cathepsin S-deficient splenocytes to present an HEL-derived peptide to a T cell hybridoma and by the presence of surface MHC class II/peptide complexes on splenocytes of -/- mice. This may explain why there were neither major defects in T cell development nor increased autoreactivity in mixed lymphocyte reactions between +/+ and -/-mice as demonstrated in Example II, as only few MHC class II/peptide complexes are required for normal maturation of thymocytes (Fukui et al. (1997) *Immunity* 7:175). This may also account for the observed IgG1 responses in -/- mice which, although depressed by approximately 50%, developed in response to either immunogen. Even so, in the absence of efficient antigen presentation, the immune responses were truncated and antibody isotypes indicative of class switching developed weakly or not at all. This scenario appears similar to that described for CD40 ligand deficiency, in which the inability of helper T cells to activate B cells by co-stimulation through CD40 results in defective germinal center formation and defective IgG class switching (Xu et al. (1994) *Immunity* 1:423). In addition to co-stimulation, efficient Ii processing within B cell-rich lymphoid follicles is critical to their development and function. Strong similarities between the consequences of cathepsin S deficiency and defects in B cell co-stimulation also raise the possibility that B cells are the cells most critically affected in the systemic defect in antigen presentation displayed by

cathepsin S-deficient mice. It is thus possible that regulation of cathepsin S activity through specific inhibitors might attenuate high titers of harmful autoantibodies or IgE without causing deleterious immunosuppression.

Other embodiments are within the following claims.

What is claimed:

1. A method of modulating autoimmunity in a subject comprising administering to the subject an agent which modulates cathepsin S activity.

2. The method of claim 1, wherein the cathepsin S activity is proteolysis of the Ii chain.

3. The method of claim 2, wherein autoimmunity in the subject is inhibited by inhibiting cathepsin S activity.

4. The method of claim 3, wherein an agent which inhibits cathepsin S activity is administered to the subject.

5. The method of claim 4, wherein the agent which inhibits cathepsin S activity is selected from the group consisting of an antisense cathepsin nucleic acid molecule, a cathepsin-specific antibody and a cathepsin inhibitory molecule.

6. The method of claim 5, wherein the cathepsin inhibitory molecule is a peptide based on vinylsulfone.

7. The method of claim 1, wherein autoimmunity in the subject is increased by increasing cathepsin S activity.

8. The method of claim 7, wherein an agent which increases cathepsin S activity is administered to the subject.

9. The method of claim 8, wherein the agent which increases cathepsin S activity is selected from the group consisting of a cathepsin S, a protein nucleic acid molecule which encode cathepsin S, and portions thereof.

10. The method of claim 1, wherein autoimmunity is modulated by modulating MHC class II-dependent immunity.

11. A method of inhibiting autoimmunity in a subject comprising administering to the subject an agent which inhibits cathepsin S activity.

12. The method of claim 11, wherein the cathepsin S activity is proteolysis of the Ii chain.

13. The method of claim 12, wherein the agent which inhibits cathepsin S activity is an antisense cathepsin nucleic acid molecule.

14. The method of claim 12, wherein the agent which inhibits cathepsin S activity is a cathepsin-specific antibody.

15. The method of claim 12, wherein the agent which inhibits cathepsin S activity is a cathepsin inhibitory molecule.

16. The method of claim 15, wherein the cathepsin inhibitory molecule is a peptide based on vinylsulfone.

17. A method of modulating isotype switching in a subject comprising administering to a subject an agent which modulates cathepsin S activity such that Ii chain proteolysis is modulated.

18. The method of claim 17, wherein isotype switching in the subject is inhibited by inhibiting cathepsin S activity.

19. The method of claim 18, wherein an agent which inhibits cathepsin S activity is administered to the subject.

20. The method of claim 19, wherein the agent which inhibits cathepsin S activity is an antisense cathepsin nucleic acid molecule.

21. The method of claim 19, wherein the agent which inhibits cathepsin S activity is a cathepsin-specific antibody.

22. The method of claim 19, wherein the agent which inhibits cathepsin S activity is a cathepsin inhibitory molecule.

23. The method of claim 22, wherein the cathepsin inhibitory molecule is a peptide based on vinylsulfone.

24. The method of claim 17, wherein isotype switching in the subject is increased by increasing cathepsin S activity.

25. The method of claim 24, wherein an agent which increases cathepsin S activity is administered to the subject.

26. The method of claim 25, wherein the agent which increases cathepsin S activity is a cathepsin S protein or a portion thereof.

27. The method of claim 25, wherein the agent which increases cathepsin S activity is a nucleic acid molecules which encodes cathepsin S, or a portion thereof..

28. A method of treating an autoimmune disorder in a subject comprising administering to a subject having an autoimmune disorder an agent which inhibits cathepsin S activity, to thereby treat the autoimmune disorder.

29. The method of claim 28, wherein the autoimmune disorder is associated with high titers of harmful autoantibodies.

30. The method of claim 29, wherein the autoimmune disorder is asthma.

31. The method of claim 28, wherein the agent which inhibits cathepsin S activity is an antisense cathepsin nucleic acid molecule.

32. The method of claim 28, wherein the agent which inhibits cathepsin S activity is a cathepsin-specific antibody.

33. The method of claim 28, wherein the agent which inhibits cathepsin S activity is a cathepsin inhibitory molecule.

34. The method of claim 33, wherein the cathepsin inhibitory molecule is a peptide based on vinylsulfone.

35. A method of evaluating a treatment for its ability to modulate an immune response comprising evaluating the ability of a compound to modulate cathepsin S activity.

36. The method of claim 35, wherein the compound can be evaluated by determining the presence or absence of the Ii chain on the surface of a cell.

37. The method of claim 36, wherein the presence or absence of Ii on the cell surface is determined using a labeled anti-Ii specific antibody.

38. The method of claim 35, wherein the treatment is evaluated for its ability to inhibit an immune response.

39. The method of claim 38, wherein the immune response is an MHC class II-dependent response.

40. The method of claim 38, wherein the presence of Ii on a cell surface is determines the ability of a compound to inhibit cathepsin S activity.

41. The method of claim 40, wherein the presence of Ii on a cell surface is determined using a labeled anti-Ii specific antibody.

42. The method of claim 35, wherein the treatment is evaluated for its ability to increase an immune response.

43. The method of claim 42, wherein the immune response is an MHC class II-dependent response.

44. The method of claim 42, wherein the absence of Ii on a cell surface determines the ability of a compound to increase cathepsin S activity.

45. The method of claim 44, wherein the absence of Ii on a cell surface is determined using a labeled anti-Ii specific antibody.

46. The method of claim 44, wherein the cell expresses Ii on the cell surface prior to the treatment.

47. A method of diagnosing a subject at risk for an autoimmune disorder comprising evaluating the presence of the Ii chain on a cell surface to thereby diagnose the subject.

48. The method of claim 47, wherein the autoimmune disorder is an enzyme deficiency disorder.

49. The method of claim 48, wherein the enzyme deficiency disorder is a cathepsin S deficiency.

50. The method of claim 49, wherein the presence of the Ii chain on a cell surface is an indicator of a cathepsin S deficiency in a subject.

51. The method of claim 47, wherein the presence of the Ii chain is determined using a labeled anti-Ii antibody.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/10100

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 48/00, 35/00, 39/395; C12Q 1/68
US CL : 514/2, 44; 435/6, 7.1; 424/130.1, 138.1, 141.1, 146.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/2, 44; 435/6, 7.1; 424/130.1, 138.1, 141.1, 146.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE, EMBASE, SCISEARCH, CAPLUS, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	RIESE et al. Cathepsin S Activity Regulates Antigen Presentation and Immunity. J. Clin. Invest. June 1998, Vol. 101, No. 11, pages 2351-2363, see entire document.	1-51
A	MORTON et al. Delivery of Nascent MHC Class II-Invariant Chain Complexes to Lysosomal Compartments and Proteolysis of Invariant Chain by Cysteine Proteases Precedes Peptide Binding in B-Lymphoblastoid Cells. J. Immunology. 1995, Vol. 154, No. 1, pages 137-150, see entire document.	1-51

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"B"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

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